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INFECTION OF NEW HOSTS BY *ANOPLODIUM HYMANAE*, A TURBELLARIAN FLATWORM (NEORHABDOCOELA, UMAGILLIDAE) INHABITING THE COELOM OF THE SEA CUCUMBER *STICHOPUS CALIFORNICUS*

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Abstract

Anoplodium hymanae, a member of the turbellarian family Umagillidae, parasitizes the holothuroid *Stichopus californicus* in the N. E. Pacific. Experiments revealed that the life cycle is direct, and that encapsulated embryos are the infective stage. Embryogenesis may or may not be completed by the time egg capsules pass out of the host of the parent worm. Developed embryos can survive in their capsules for 10–11 months, but they die if the capsules remain in sea water indefinitely. Hatching occurs when egg capsules that contain developed embryos are ingested by a sea cucumber; hatching is induced by the host digestive fluids. Larvae reach the coelom by penetrating the wall of the lower intestine or, more commonly, the wall of the respiratory trees. Larvae of *A. hymanae* and those of intestine-inhabiting umagillids differ in behavior, but appear similar in morphology. The size of worms infesting *S. californicus* varies seasonally and is correlated with the seasonal feeding behavior of the host.

Introduction

Most species of turbellarian flatworms are free-living, but more than 150 species belonging to more or less distantly related families have evolved symbiotic associations with other organisms (reviewed by Jennings, 1971; Shinn, 1984). The hosts are typically invertebrates. Symbiotic turbellarians may inhabit the outside surface, the digestive tract, the connective tissue, or the body cavity of the host. As far as is known, symbiotic turbellarians have simple life cycles that do not involve intermediate hosts. The life history has been elucidated for remarkably few symbiotic turbellarians, however (Jennings, 1971; Shinn, 1984). The life history is known for only one turbellarian that inhabits the body cavity of its host. That species, *Kronborgia amphipodicola*, belongs to the neorhabdocoel family Fecampiidae, and like most other fecampiids, inhabits crustaceans. The worms leave the host after attaining maturity. Females secrete a cocoon into which a large number of egg capsules are deposited. Free-swimming larvae hatch out, locate a prospective host, attach to the outside of it, secrete a cyst around themselves, and then bore through the body wall (Christensen and Kanneworff, 1965).

From information presented in taxonomic papers, it is evident that the reproductive biology of other groups of coelom-inhabiting turbellarians differs from that of the fecampiids. Members of other taxa typically produce and release egg capsules during the parasitic phase (Jennings, 1971, and references therein).

The largest of the families of symbiotic turbellarians is the neorhabdocoel family Umagillidae. These flatworms inhabit echinoderms and sipunculans (see Cannon,

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1982, for systematic review). Most of the 50+ described species of umagillids live in the intestine of the host, but ten species are reported to live in the intestine and coelom of sea urchins or sea cucumbers, and eight species occur exclusively in the coelom of sea cucumbers. All of the latter eight species belong to the genus *Anoplodium*.

Schneider (1858) reported that egg capsules of *Anoplodium parasita* are laid into the coelom of its hosts, *Holothuria* spp. He predicted that the egg capsules escape the coelom before hatching because egg capsules that he recovered from the coelom were never hatched. Schneider's hypothesis is correct for the related species *A. hymanae* (Shinn, 1985). Egg capsules of *A. hymanae* pass out of the body cavity of its host, *Stichopus californicus*, on a daily basis, apparently through ducts that connect the perivisceral coelom to the posterior end of the rectum. Egg capsules and adult or subadult worms are also cast out of the coelom when the hosts eviscerate. Thus, egg capsules and free-moving worms are potential infective stages for *A. hymanae*. Schneider (1858) hypothesized that embryos in egg capsules of *A. parasita* would hatch in the sea. Apparently he thought that free-swimming larvae penetrate new hosts, although he did not propose a site of penetration. Changeux (1961) presented the alternative hypothesis that encapsulated embryos of *A. parasita* are ingested by a new host, and that the embryos hatch in the intestine, then penetrate the wall of the intestine to reach the coelom.

This paper describes the mechanism of infection of new hosts by the umagillid *Anoplodium hymanae* Shinn. This flatworm and its host (*Stichopus californicus*) occur along the western coast of North America (Shinn, 1983a). The hypotheses of Schneider (1858) and Changeux (1961) concerning the mechanism of infection were tested experimentally, as was the hypothesis that free-moving worms that are expelled at evisceration can infect new hosts. The morphology of larvae is described. In addition, this study has revealed a distinct variation in the size of worms present in *S. californicus*. The variation is correlated with the seasonal feeding behavior of the host.

Materials and Methods

Collecting sites, procedures for dissecting sea cucumbers, and the protocol for keeping worms alive outside of the host are described by Shinn (1985).

Maintenance of egg capsules to determine if embryos hatch spontaneously

Egg capsules that had been expelled by natural means from *Stichopus californicus* (see Shinn, 1985) were kept in loosely capped jars of filtered sea water. Most of the capsules were teased out of the masses of coelomocytes in which they had been expelled. The sea water was changed every 2–3 days. Approximately 400 large capsules (*i.e.*, the size produced by large worms) were kept in constant darkness, and about 400 large capsules were kept in an uncontrolled light/dark regimen. A separate batch of about 200 small capsules (the size produced by small worms) were also kept in an uncontrolled light/dark regimen. Subsamples of 20–50 capsules were examined with a compound microscope every week for the first two months, and then only every several weeks. Capsules in some of the subsamples were used in experiments concerning the stimulus for hatching (see below). During microscopic studies, coverglasses were supported with pieces of clay so that the embryos were not damaged.

Infection of new hosts by ingestion of egg capsules

Small *Stichopus californicus* (less than 5 cm long) were collected intertidally from Shaw Island, Washington (48°36′10″N; 122°48′80″W), where they had apparently set-

tled as larvae and were growing up out of contact with adult *S. californicus*. Several specimens were dissected and found to be uninfested by *A. hymanae*. Consequently, two other specimens were used in experiments to test the hypothesis that new hosts are infected when they ingest capsules containing larvae. The experimental sea cucumbers were kept for several weeks in dishes of filtered sea water. They were fed a cereal-type baby food to sustain them and flush the digestive tract of inorganic debris. Periodic examination of the water in which they were kept revealed no egg capsules. Several dozen fully embryonated egg capsules of *A. hymanae* were added to the dishes over a period of 24 h. As feces were deposited, they were collected and examined for egg capsules. The sea cucumbers were dissected at the end of 48 h. They were rinsed thoroughly with fresh water, sliced open with a razor blade, and the coelom was flushed with filtered sea water. Washings from the coelom were examined for *A. hymanae*. The digestive tract/respiratory tree complex was excised and fixed in hot (60°C) Hollande's fixative, embedded in paraffin, and sectioned at 8 μm. Sections were stained with Weigert's hematoxylin and erythrosin B.

Site and cause of hatching

Fifty to seventy-five fully embryonated capsules of *Anoplodium hymanae* were pipetted into short pieces of the intestine of large *Stichopus californicus*. The pieces were tied at both ends in order to contain the digestive fluids. Two pieces were taken from the upper third of the intestine, which is characterized by a highly folded wall, and two were taken from the lower third of the intestine, which has a non-folded wall. The pieces of intestine were kept in autoclaved sea water and opened after 7 and 30 hours. After examination, the tissues were fixed, embedded in paraffin, and sectioned as described above. Similar results were obtained for the two durations, so the data were combined. The pieces of intestine were intact after seven hours, but the digestive epithelium had deteriorated by 30 hours.

Two sets of experiments were performed to determine whether hatching results from the direct action of the digestive fluids of *Stichopus californicus* on the opercular sutures of the egg capsules of *Anoplodium hymanae:* (1) intact capsules that had been treated to kill the enclosed embryos were submerged in digestive fluids from the upper, folded part of the intestine of *S. californicus*. The capsules had been collected after their natural release from the coelom and were kept in sea water until the embryos had completed development. The embryos were then killed by placing the capsules in a small amount of sea water and letting the water evaporate to about half its original volume. This required about 24 h. Some capsules became indented during the process, but they eventually returned to their original form. Before the experiments, the capsules were returned to normal sea water for several hours. They were examined with a compound microscope to verify that the embryos were dead and that the opercular sutures were intact. (2) Fully embryonated capsules were sliced open with a razor blade and the ends of the capsules that contained the intact opercular suture were immersed in digestive fluids of *S. californicus* as described above. To control for the effectiveness of the digestive fluids as "causing" hatching, capsules containing live, developed embryos were submerged in aliquots of the digestive fluids. The experiments were conducted at 10°C. The capsules were examined after 1 h and again after 18 h.

Infection of hosts by adult worms

Experiments to determine whether adult worms can infect new host are described in the Results section. The experimental sea cucumbers were kept in separate aquaria,

without running sea water, so that worms introduced to the sea cucumbers could be recovered and counted. Although the objective was to determine if worms released at evisceration might be able to infect new hosts, worms used in the experiments were initially dissected from sea cucumbers. Unless sea cucumbers undergo some general physiological changes just prior to evisceration, the effect of being dissected from the host should not be much different for the worms than if they had been naturally eviscerated. This procedure was considered to be more natural than evisceration induced by injecting chemicals into the perivisceral coelom.

Results

Fate of expelled egg capsules

Naturally released capsules of *Anoplodium hymanae* that were kept in sea water at local marine temperatures (8–12°C) contained developed embryos within three weeks of collection. The newly developed embryos were surrounded by a layer of yolk cells that had not been incorporated during embryogenesis.

Embryos did not hatch from either large or small capsules that were kept in sea water. This was the case for capsules kept in the dark and for those kept in an alternating light/dark regimen. Embryos in small capsules began to die after 5 months, but essentially all embryos in the subsamples of large capsules were alive after 8½ months. Approximately 80% of the large capsules contained live embryos after 10 months. After 11 months, the remaining 146 large capsules were examined: 58% (84) contained live embryos, and 42% (62) contained dead embryos. Some debris was visible in the capsules containing live embryos, but there were no recognizable yolk cells. I could not distinguish whether the yolk cells had disintegrated or had been consumed by the embryos. The walls of capsules that contained live embryos, or dead but intact embryos, were still pliable at the end of the eleven-month incubation period. The opercula could not be dislodged without destroying the capsules. Capsules that were compressed under a coverglass split lengthwise, though there was a tendency for the split to follow the suture for a short distance. Dead embryos eventually disintegrated in the capsules. The operculum of most capsules containing long-disintegrated embryos remained in place, but was usually dislodged by the slightest contact. No hatchlings were seen among any of the sets of capsules.

Infection of new hosts by encapsulated embryos

Fully embryonated capsules that were offered along with food to small, "worm-free" specimens of *Stichopus californicus* began to appear in the feces of the holothuroids after about 24 h. The feces had the form of elongate strings of detritus ensheathed by mucus. Egg capsules that had passed through the sea cucumbers were enclosed in the layer of mucus and were thus easily distinguished from capsules that had not been eaten. By the end of 48 h, 168 capsules had been recovered from the feces: 100 (60%) had the operculum dislodged and were empty; 7 (4%) were opened but still contained the embryos; 57 (34%) were closed and contained live embryos; 4 (2%) were closed but contained dead embryos. About 100 viable capsules were not ingested; none hatched.

Four small (150–220 μm long) *Anoplodium hymanae* were recovered from the perivisceral coelom of each of the two small *Stichopus californicus* to which the embryonated capsules had been fed. The worms were very hard to see because they were transparent and clung to pieces of tissue; additional small specimens may have been overlooked. In paraffin sections of the viscera of the sea cucumbers, numerous hatch-

lings of *A. hymanae* were observed among the food in the intestinal lumen. Several dozen worms in various stages of penetration through the wall of the lower part of the intestine and, especially, through the wall of the respiratory trees, were found in paraffin sections of one of the sea cucumbers (Figs. 1–4). Passage through these organs appears to occur in stages. Many specimens were located immediately beneath the luminal epithelium. This layer probably closes over the penetrating larvae very quickly because it was seldom observed to be broken. Specimens within the connective tissue layer of the respiratory trees and lower intestine were usually oriented parallel to the luminal and coelomic epithelia, with the ventral surface adjacent to the coelomic epithelium. No specimens extended completely across the wall of the respiratory trees or intestine.

Site and cause of hatching

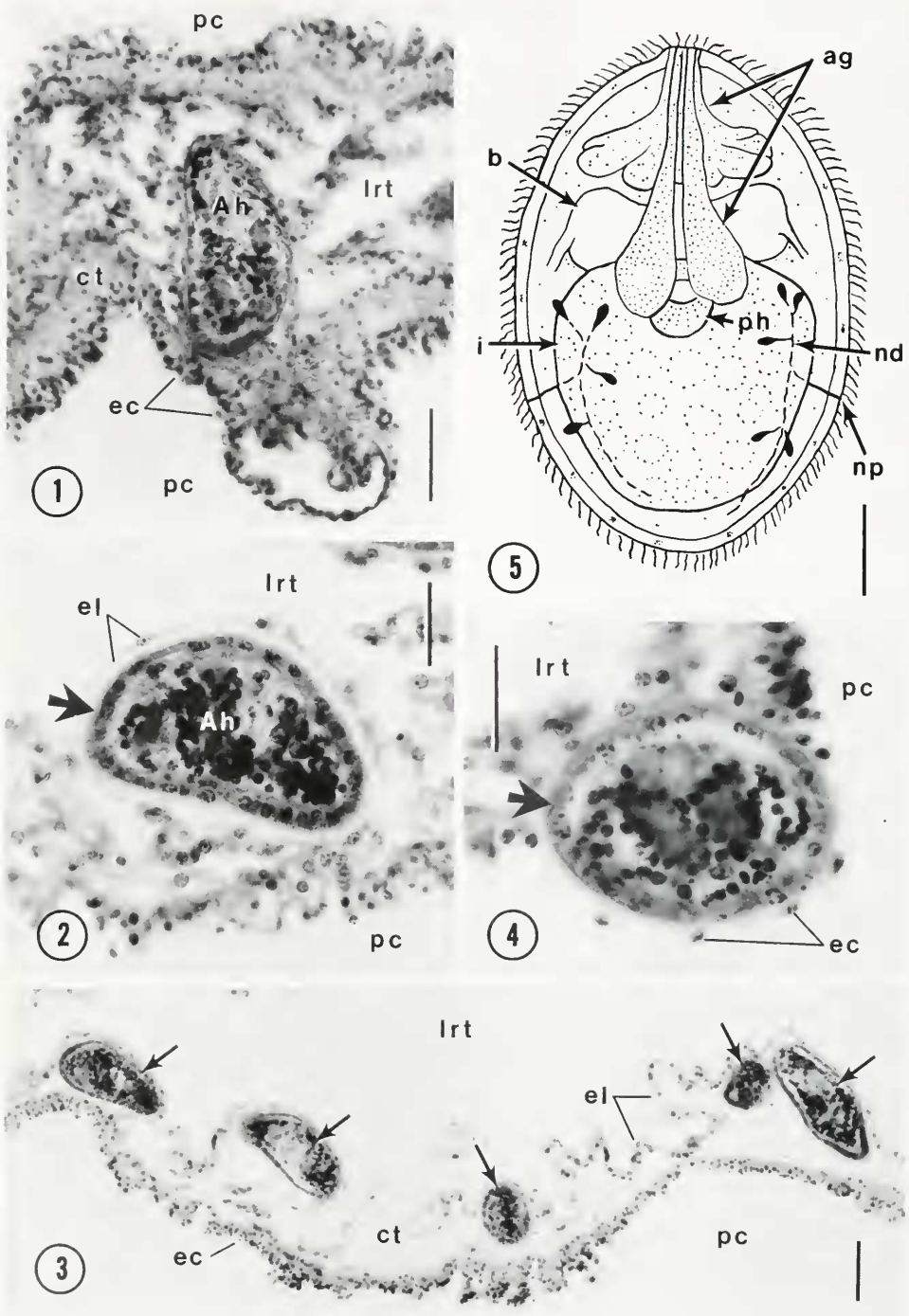
Fluid from only the anterior part of the intestine of *Stichopus californicus* is very effective at "causing" hatching of *Anoplodium hymanae*. Of 105 capsules that were introduced into tied-off pieces of the foregut of *S. californicus*, 82 (78%) had hatched, 3 (2.8%) were in the process of hatching, 17 (16%) were unhatched, and 3 (2.8%) were dead when the pieces were opened. Large numbers of larvae were recovered from the lumen of the pieces of foregut, but none was found to be penetrating the tissues when the latter were sectioned. Only two of 155 capsules introduced into pieces of hindgut hatched, though the embryos in the capsules were still alive at the end of the experiment.

Embryos hatched from 42 (86%) of 49 capsules that were immersed in fluids from the upper, folded part of the intestine of *Stichopus californicus*. These capsules constituted the controls for experiments to determine whether the opercular sutures of egg capsules are broken down by the enzymatic action of the intestinal fluids of *S. californicus* (see below). Three embryos had emerged by the end of one hour. At this time, 25 control capsules (not included in the above totals) were examined with a compound microscope: 6 had the operculum dislodged but still contained the embryo, and 19 had the operculum in place. To determine if the opercular suture of the intact capsules had become weakened in preparation for hatching, pressure was applied to the capsules by slowly drawing water out from beneath the coverglass. Slight compression resulted in the operculum being dislodged from 18 of the closed capsules; application of greater pressure caused the last capsule to split without the operculum becoming dislodged. The walls of the capsules were still pliant.

Fluids from the folded part of the intestine of *Stichopus californicus* did not weaken the opercular suture of capsules of *Anoplodium hymanae*. The experiments included 125 intact capsules whose developed embryos had been killed, and 20 capsules that were sliced open to allow the intestinal fluids to contact the inner edge of the opercular suture. The location of the opercular suture on the outer surface of the capsules was, however, more conspicuous after immersion in intestinal fluids. When the capsules were compressed after 18 h in the intestinal fluids the still-pliable capsules became deformed and eventually split without the operculum being dislodged.

Morphology of larvae

Larvae are dorsoventrally flattened and ovoid in outline when crawling over a firm surface. The body is colorless and completely ciliated. The larvae vary in size with the size of the capsule in which they develop, and with the number of zygotes that are incorporated into the capsule. The largest larvae are approximately 200 μm



FIGURES 1–4. Larvae of *Anoplodium hymanae* (Ah) in various stages of penetration through the wall of the respiratory tree of *Stichopus californicus* after experimental infection; light micrographs of paraffin sections; H & E stain. lrt, lumen of respiratory tree; el, luminal epithelium; ct, connective tissue wall of respiratory tree; ec, coelomic epithelium; pc, perivisceral coelom.

FIGURE 1. The worm is in contact at its anterior end with the luminal side of the wall of the respiratory tree. Scale = 50 μm.

long and 95 μm wide, but specimens as small as 70 μm long and 40 μm wide were seen. The epidermis consists of cuboidal cells that are approximately 5 μm tall; the cilia are about 5 μm long. The epidermal cells lack rhabdites. The epidermis remains intact during penetration of larvae into the coelom of the new host.

Newly hatched larvae contain at least four large, intensely eosinophilic gland cells which open at the anterior tip (Fig. 5; see also Shinn, 1985: Fig. 9). Two cells are positioned dorsally and extend ⅔ the length of the body. Two (possibly more) cells lie ventrally in the anterior ⅓ of the body. At least some gland cells were present in specimens that were penetrating the wall of the respiratory trees. The glands were lacking from even the smallest coelomic worms.

The cerebral ganglion lies immediately behind the ventral pair of anterior gland cells. It consists of a central bilobed neuropile surrounded by numerous cell bodies.

The mouth opens on the ventral midline about midbody. The doliiform pharynx measures about 20 μm in diameter, and is located immediately behind the brain. The pharynx leads dorsally to the intestine. The latter extends posteriorly and laterally to the edges of the body. It consists of large cuboidal cells which contain numerous, fluid-filled vacuoles, and lipoid and yolk inclusions.

The protonephridial system is arranged bilaterally near the lateral body margins. A nephridiopore is located on each side of the body just posterior to the level of the pharynx (Fig. 5). These lead into short, medially directed collecting ducts that divide into an anterior and a posterior duct. Five flame cells were visible on each side of the body; the tip of the posterior duct on each side apparently lacks a flame cell.

Can adult worms infect new hosts?

Adult *Anoplodium hymanae* that were exposed by various means to specimens of *Stichopus californicus* were never found to infect the sea cucumbers. Twenty worms were placed in aquaria that contained sea cucumbers. The worms were very active for at least several hours, but were all dead at the end of 24 h. They had been mutilated, apparently by the feeding activities of the sea cucumbers. Twenty worms were pipetted onto the dorsal epidermis of *S. californicus;* they quickly crawled off the holothuroids and eventually died on the bottom of the aquaria. Twenty *A. hymanae* were pipetted into the mouths of two *S. californicus;* the worms were necrotic when dissected out of the intestine after two hours. When specimens of *A. hymanae* were placed directly into digestive fluids that had been freshly removed from the anterior part of the intestine of a sea cucumber, they underwent several spasmodic contractions and died within seconds. Twenty worms introduced into the rectum of *S. californicus* via the inhalent current were expelled within an hour. The worms died on the bottom of the aquaria.

Intensity and seasonality of infestation

A census of *Anoplodium hymanae* infestations of 13–15 *Stichopus californicus* was made at five widely separated times during two consecutive annual feeding cycles

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FIGURE 2. Worm is located just beneath the luminal epithelium. The epidermis (arrow) of the larva is intact. Scale = 25 μm.

FIGURE 3. Sections through five worms (arrows), all located in the connective tissue compartment. Scale = 50 μm.

FIGURE 4. Worm (arrow) is located subjacent to the coelomic epithelium just before breaking out into the coelom. Scale = 25 μm.

FIGURE 5. Hatchling of *A. hymanae;* free-hand drawing from photographs and live specimens; dorsal view. ag, anterior glands; b, brain; i, intestine; np, nephridiopores; ph, pharynx; nd, protonephridial ducts. Scale = 25 μm.

of the host (Table I). During the first sampling period only the number and relative sizes of worms and the status of the host digestive tract was recorded in a systematic way. Subsequently, the lengths and reproductive status of the worms, and the number of egg capsules in the brown bodies were also recorded.

All *Stichopus californicus* examined during the first, third, and fifth sampling periods (*i.e.*, during the springs of 1981 and 1983 and summer of 1982) had fully differentiated digestive tracts filled with food. *Stichopus californicus* dissected during the second sampling period (during the fall of 1981) had either a degenerating intestine (lumen indistinct or filled with cellular debris) or a non-functional, regenerating intestine (lumen narrow and empty, regions of intestine not differentiated). Sea cucumbers dissected during the fourth sampling period (early winter of 1982) had a non-functional regenerating intestine or a regenerating intestine that contained a thin column of food. According to these observations, *S. californicus* does not feed between about early October and early December. Specimens in the population do not all cease and recommence feeding on the same dates, however. Only one specimen, which was collected during the fall of 1981 but which was not part of the *A. hymanae* census, had obviously eviscerated its digestive tract in the field.

Seventy-two of the 75 *Stichopus californicus* that were examined during the five sampling periods contained *Anoplodium hymanae* (Table I). The infested *S. californicus* contained a mean of 25.5 (range 1–147) worms. At all times of the year there was great variation in the intensity of infestation between hosts (Table I). The median number of worms per host was greater during the summer than fall of 1981, but the numbers of worms were not significantly different at the $P = 0.05$ level (Wilcoxon rank sum test). Conversely, the median number of worms per host was smaller during the summer than fall of 1982, though the numbers of worms were not significantly

TABLE I

Intensity of infestation of Stichopus californicus *with* Anoplodium hymanae *at different sampling periods*

Sampling period & date	Condition of intestines of *Stichopus californicus*	Percent of *Stichopus californicus* infested (no. examined)	Mean no. *Anoplodium hymanae* per host	Range
1. 9–24 June 1981	Functional, fully differentiated	93.7% (16)	17.5	0–52
2. 12 Oct. to 27 Nov. 1981	Non-functional	87% (16)	9	0–34
3. 23 Aug. 1982	Functional, fully differentiated	100% (15)	17.8	1–43
4. 4 Dec. 1982	Non-functional	100% (8)	26.4	1–106
	Functional, regenerating	100% (7)	52.7	1–133
5. 14 Apr. 1983	Functional, fully differentiated	100% (13)	44	4–147
Totals		96% (75)	25.5	0–147

different at the $P = 0.1$ level (Wilcoxon rank sum test). Over the entire study, hosts with a fully differentiated gut contained more worms than did hosts with a non-functional gut ($P < 0.05$, Wilcoxon rank sum test).

It was noted in an earlier paper that *Stichopus californicus* often contains two conspicuously different sizes of *Anoplodium hymanae* (Shinn, 1983a). This was the case for most hosts dissected during the spring and early summer (Table II). Each size class actually consisted of worms of a range of sizes; worms of intermediate sizes were generally absent (Fig. 6). Other *S. californicus* dissected during the five sampling periods contained worms within only a certain range of small sizes (Table II; Fig. 7). The percentage of hosts that contain only small worms apparently increases as the feeding periods progress so that during the late fall and early winter, *S. californicus* contain only small *A. hymanae* (Table II).

Seven *Stichopus californicus* that were dissected during August 1982 contained only small worms. Six of the seven contained capsules of the size produced by large worms. The embryos in many of the large capsules had not completed embryogenesis. Because the duration of the embryogenic period is 30–35 days (Shinn, 1985), the hosts must have contained large worms less than 35 days prior to dissection. Similarly, brown bodies in 7 of the 16 hosts dissected during October and November 1981, and 9 of the 15 hosts dissected during December 1982, contained capsules of the size produced by large worms, while at the time of dissection the hosts harbored only small worms. Most of the capsules contained viable, developed embryos. These observations provide further evidence that most hosts are inhabited by large and small worms early in the year, and that the large worms disappear from all hosts by the end of fall.

Over both years of this study, the mean number of egg capsules recovered from the coelom per host was greater during the spring and summer than in the late fall and winter (Table III). While large worms were absent from sea cucumbers during the winter, at least some small worms in some hosts were reproductive during the winter. Egg capsules are produced and, presumably, released to the environment for infection of new hosts at all times of the year.

TABLE II

Occurrence of large and small specimens of Anoplodium hymanae *in* Stichopus californicus *at different times of year**

Date**	Number of *S. californicus* examined	Percent of *S. californicus* infested with both large and small worms	Percent of *S. californicus* infested only with small worms	Percent of *S. californicus* that contained large capsules but no large worms
April (1983)	13	100%	0%	nd
June (1981)	16	63%	25%	nd
August (1982)	15	53%	47%	40%
Oct.–Nov. (1981)	16	0%	87%	43%
Dec. (1982)	15	0%	100%	60%

* See *Intensity and seasonality of infestation* section for detailed explanation of size classes of worms.

** Note that sampling dates from different years are arranged in a monthly sequence.

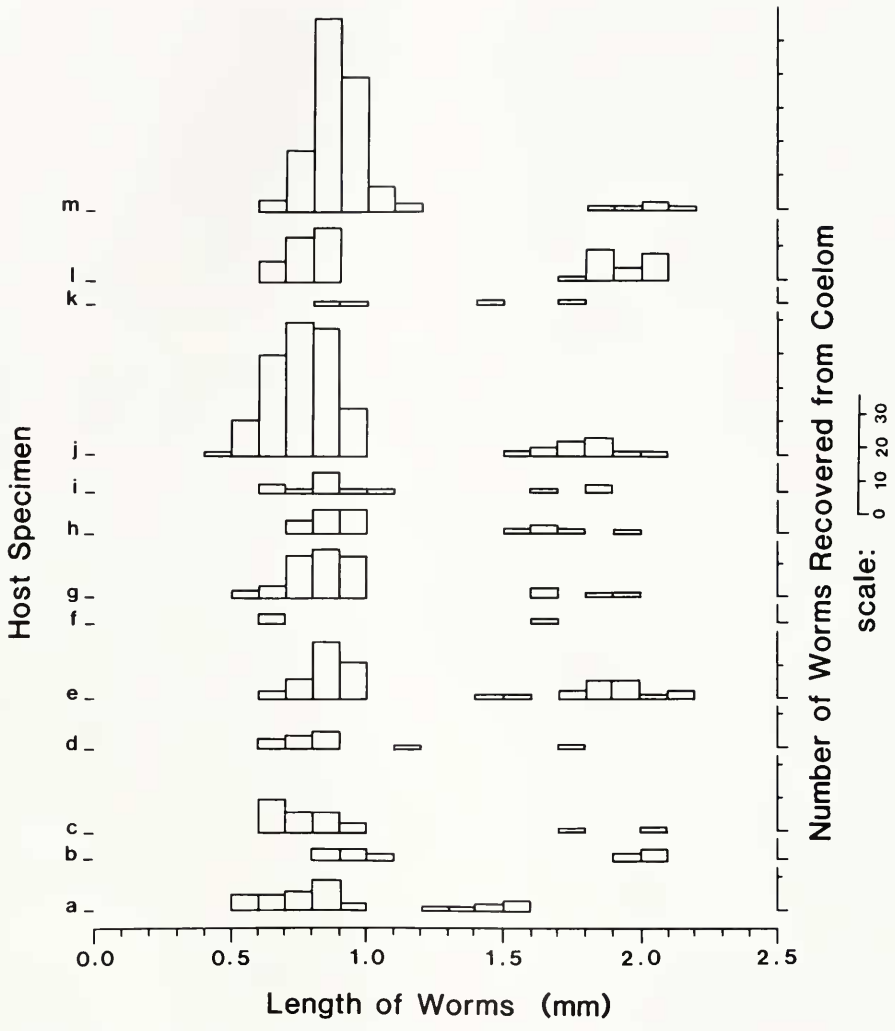

FIGURE 6. Histograms showing numbers of *Anoplodium hymanae* (ordinate) of various lengths (abscissa) recovered from each of 13 *Stichopus californicus* (a–m) on 14 April 1983. Most hosts contained worms of two size ranges, and lacked worms of an intermediate size range.

DISCUSSION

Infection of new hosts

It can be concluded from experiments and observations described in this paper that egg capsules containing developed embryos are the usual infective stage of the life history of *Anoplodium hymanae*. In contrast to Schneider's (1858) hypothesis, embryos do not normally hatch from capsules that remain in sea water. They hatch when the capsules are ingested by a potential host, as hypothesized by Changeux (1961).

Stichopus californicus is an epibenthic species. Like most other aspidochirote holothuroids, it consumes surface detritus by the use of its peltate, oral tube feet

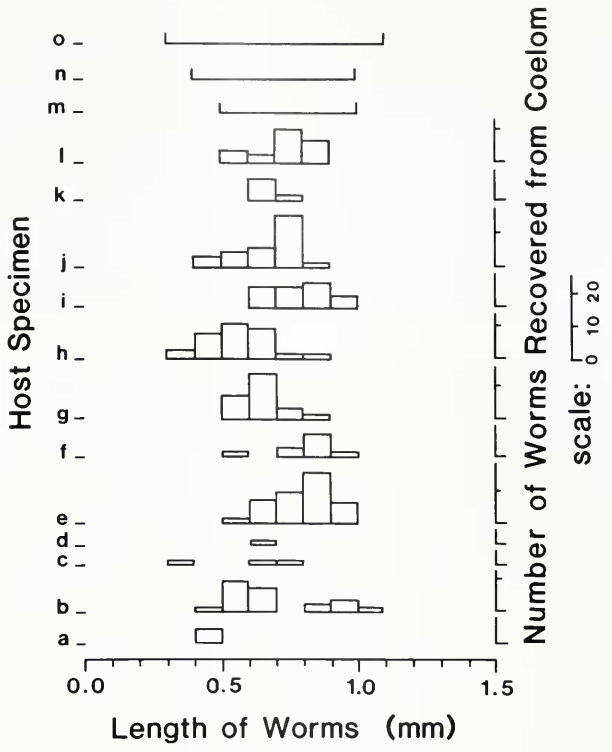

FIGURE 7. Histograms showing numbers of *Anoplodium hymanae* (ordinate) of various lengths (abscissa) recovered from each of 15 *Stichopus californicus* collected on 5 December 1982. Only the size range of worms are shown for hosts m, n, and o; they contained 122, 105, and 119 worms, respectively. The hosts contained only small worms.

(Massin, 1982; Cameron and Fankboner, 1984). Particles that stick to mucus on the feeding appendages are transferred to the mouth. Food particles might also become entrapped between small knobs of the oral tube feet (Cameron and Fankboner, 1984). The latter mechanism may provide some selectivity in feeding (Roberts, 1979). Egg capsules of *Anoplodium hymanae* are probably ingested at random along with detritus, although the long stalk on the capsules (see Shinn, 1985) may facilitate ensnarement of the capsules.

TABLE III

Seasonal abundance of egg capsules of Anoplodium hymanae *in brown bodies of* Stichopus californicus

Sampling period & dates	Total number hosts examined	Mean number capsules per host	Range in number capsules per host
1. 9–24 June 1981	1	763	—
2. 12 Oct. to 27 Nov. 1981	11	82	0–368
3. 23 Aug. 1982	15	487	2–3327
4. 4 Dec. 1982	14	287	0–1520
5. 14 Apr. 1983	13	969	161–2156

Twenty-seven of the 28 species of umagillids that have been described from holothuroids inhabit members of the order Aspidochirota (Cannon, 1982; Shinn, 1983a). The other species and an undescribed umagillid are reported from members of the holothuroid order Apoda (Barel and Kramers, 1970; Kawakatsu, 1983). Members of the Aspidochirota and Apoda are typically sediment-ingesting detritivores (Massin, 1982). No umagillids are known to inhabit dendrochirote holothuroids, which are typically suspension feeders. This restriction of both intestine- and coelom-inhabiting umagillids of holothuroids to detritivorous hosts suggests that the infective stages of these various species of worms are, like the egg capsules of *A. hymanae,* ingested along with detritus from the sea floor. The fact that *A. hymanae* reaches the body cavity via the digestive tract suggests that species of *Anoplodium* have evolved from intestine-inhabiting ancestors.

Developed embryos of *Anoplodium hymanae* hatch upon contacting digestive fluids in the foregut of *Stichopus californicus.* My experiments have revealed that the opercular suture of the egg capsules is not broken down by the digestive enzymes of the host. I conclude that weakening of the opercular suture results from the activities of the embryos themselves, and that the embryos are induced to hatch by the digestive fluids. Hatching of the intestine-inhabiting umagillid *Syndisyrinx franciscanus* is also apparently induced by the digestive fluids of its echinoid hosts (Shinn, 1983b). The identity of the hatching stimuli of these umagillids has not been investigated. *Anoplodium hymanae* and *S. franciscanus* are, in my experience, entirely host specific, yet hosts containing the two worms co-occur at the sites where animals for this study were collected. Investigation of the species specificity of the hatching stimuli could provide considerable insight into the mechanism by which host specificity is established. Unless the hatching stimuli are very specific, ingestion of egg capsules by non-host organisms, including animals other than echinoderms, would have adverse effects on the reproductive success of these umagillids.

The time between initial contact with fluids from the foregut of *Stichopus californicus* and emergence of the larvae varies considerably. Hatching probably occurs at various levels of the gut of new hosts. The larvae must burrow out of the column of food in the gut before they can penetrate into the coelom. Embryos hatching in small sea cucumbers reach the coelom mainly by passing through the wall of the respiratory trees. Most worms hatching in large hosts may bore through the wall of the lower intestine before reaching the level of the respiratory trees.

Glands opening at the anterior end of the oncosphere larva of the cestode *Hymenolepis diminuta,* and the oncomiracidium larva of the monogenean *Entobdella soleae* apparently secrete hatching enzymes (Holmes and Fairweather, 1982; Kearn, 1975, respectively). Similarly positioned glands in the larvae of the fecampiid turbellarian *Kronborgia amphipodicola* (Køie and Bresciani, 1973), the larva of the cestodarian *Austramphilina elongata* (Rohde and Georgi, 1983), and the miracidium of the digeneans *Fasciola hepatica* (Dawes, 1960; Wilson *et al.,* 1971) and *Fascioloides magna* (Coil, 1981) are thought to produce lytic secretions that aid in penetrating the tissues of their hosts. The anterior glands of *Anoplodium hymanae* are not obviously expired before or during hatching; if a hatching enzyme is secreted by this species, the anterior glands are probably not its source. The glands may secrete a lytic substance that aids in escape from the layer of mucus that ensheaths the food in the host intestine, or that aids in penetration into the coelom. Newly-hatched specimens of the intestine-inhabiting umagillid *Syndisyrinx franciscanus* also have anterior glands that disappear soon after hatching, but those worms do not bore through the tissues of their echinoid hosts (Shinn, 1983b). This suggests that additional functions exist for the anterior glands of umagillids.

Intensity and seasonality of infestation

The lack of worms of an intermediate range of sizes during the spring and summer reveals that there is a period during which *Stichopus californicus* is not being infected by *Anoplodium hymanae*. The cessation of feeding by *S. californicus* during the fall and early winter may cause the gap in sizes of worms. Small worms that are present before feeding stops presumably grow during the non-feeding period and would be considerably larger than the new worms of the next year. The magnitude of the gap in worm sizes will reflect the duration of the nonfeeding period plus the length of time between recommencement of feeding and ingestion of infective capsules. Additional gaps in sizes of worms will result from ingestion of egg capsules at widely separated times.

Because large specimens of *Anoplodium hymanae* are not present in *Stichopus californicus* during the late fall and early winter when hosts are not feeding, it can be concluded that the large worms grow up from small worms within a single year—presumably from the small worms of the previous feeding season. Death of large worms probably begins as early as June because all hosts dissected in April 1983 had large worms, and 5 of 16 hosts dissected in June 1981 lacked large worms. The recovery of much greater numbers of small worms (up to 133) than large worms (up to 22) from individual hosts suggests that there is also heavy mortality of small worms.

The lack of large worms during the winter cannot necessarily be explained merely by senescence of the worms that grew up during the previous fall nonfeeding period. Since egg capsules are released to the environment at all times of the year, *Stichopus californicus* can be infected at any time during the feeding period. Some small worms will be acquired from about December through early October. Because large worms are found as early as mid-April, small worms can apparently attain the large size in about seven months (Oct.–Apr.). Worms acquired early during the feeding period should be just attaining full size by the following nonfeeding period. Growth of the worms may be non-linear over time (*i.e.* there may be a limit on the growth of small worms acquired during a particular feeding period) or there may be a selective die off of all remaining large worms prior to the non-feeding season. The former hypothesis is perhaps more likely because the size range of small worms remains fairly constant through the spring and summer (*cf.* Figs. 6, 7).

Development and hatching of egg capsules in the coelom

Stichopus californicus may provide a variety of brooding services to *Anoplodium hymanae*. It is conceivable that encapsulated embryos take up nutrients from the coelomic fluid of the host. No studies of the permeability of umagillid egg capsules have been made, but the wall of egg capsules of some other parasitic platyhelminths is known to be permeable to small molecules including some amino acids and carbohydrates (Rowan, 1962; Wilson, 1967).

Some embryos of *Anoplodium hymanae* may hatch and mature in the coelom. While this would be difficult to demonstrate, its possibility is suggested by the observation of four embryos hatching from capsules that were freshly collected from the coelom by dissection, and by the recovery of some open capsules from the coelom. Alternatively, the embryos in the opened capsules may have died without hatching, the opercular sutures may have subsequently broken down and the capsules may have been cleaned out by host coelomocytes.

Embryos in many capsules complete development in the coelom and are infective as soon as they are released to the sea. This may reduce exposure of the capsules to various destructive phenomena during the relatively long developmental period during

which the capsules are not infective. In contrast, the encapsulated embryos of *Syndisyrinx franciscanus* must complete their two-month embryogenic period in the sea before they are infective (Shinn, 1983b).

General conclusions

The life cycle of *Anoplodium hymanae* (Fig. 8) resembles that of the intestine inhabiting umagillid *Syndisyrinx franciscanus* in having fully embryonated capsules as the infective stage, and in having a simple life cycle (*i.e.*, no intermediate hosts are required; Shinn, 1983b). As far as is known, escape of infective stages of *A. hymanae* from the coelom depends upon the defense mechanisms of the host rather than specific adaptations of the parasite. The only major difference in the reproductive biology of

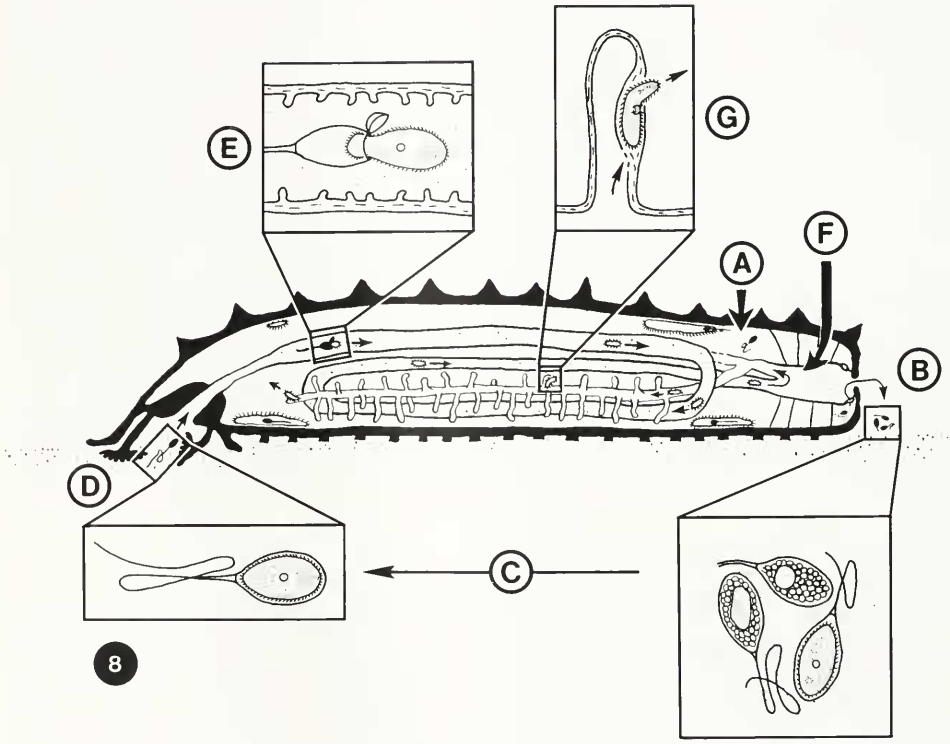

FIGURE 8. Life history of *Anoplodium hymanae*. A. *Anoplodium hymanae* releases egg capsules into the perivisceral coelom of the host. B. Capsules are encapsulated by host coelomocytes and collected into large masses called brown bodies. Brown bodies accumulate among suspensors of the rectum, then pass through ducts in the wall of the host's rectum and out the anus to the sea. At the time of release, encapsulated embryos are in various stages of development. C. Embryos complete development outside the host; developed embryos will survive in the capsules for many months but will not hatch if they remain in sea water. D. Egg capsules containing developed embryos are ingested as *Stichopus californicus* feeds on epibenthic detritus. E. Larvae hatch in response to digestive fluids in the upper intestine of the host; hatchlings escape the column of mucus-ensheathed detritus as it passes down the intestine. F. Larvae ascend the respiratory trees where the latter join the intestine. G. Larvae penetrate the wall of the respiratory trees and enter the perivisceral coelom of the new host.

these species that is associated with the difference in site of infestation is the ability of hatchlings of *A. hymanae* to penetrate the tissues of the host.

This study emphasizes that *Anoplodium hymanae* is fairly limited in the site of infestation in the host. Hatchlings of *A. hymanae* are not adversely affected by the digestive fluids of the host, but adult worms are killed by them. In addition, *A. hymanae* appears to have some means of avoiding attack by host coelomocytes. A fairly large number of species of umagillids are reported to inhabit both the coelom and intestine of the host (reviewed by Cannon, 1982; Shinn, 1984). Those cases should be carefully re-examined to determine if the worms clearly are adapted to inhabiting very different sites in their hosts, or whether the reports are the result of improper dissection techniques.

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